IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF :

EISHUN TSUCHIDA ET AL. : EXAMINER: CARR, DEBORAH D.

SERIAL NO: 10/782,851 :

FILED: FEBRUARY 23, 2004 : GROUP ART UNIT: 1621

FOR: ZWITTERIONIC LIPID COMPOUND AND USES THEREOF

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Keitaro Sou, a national of Japan, declare as follows.

I am a co-applicant of the above-identified application.

The following Experiments were conducted by me or under my direct supervision:

Experiments

A mixture of 150 mg (0.207mmol) of Compound 3 described at page 25 of the present specification, 80 mg (0.207 mmol) of cholesterol, and 29 mg (0.041 mmol) of dipalmitoylphosphoglycerol (DPPG) was placed in a 100 mL egg-plant type flask, and 10 mL of benzene was added thereto while heating mixture to completely dissolve the mixture. The resultant mixture in the flask was frozen with dry ice-methanol, and the flask was set a freeze-dry apparatus in which the mixture

was freeze-dried for 10 minutes. To the resultant freeze-dried material, 25 mL of water for injection containing NaOH in the same molar amount as the DPPG, and the mixture was stirred with a magnet stirrer to disperse the material in the aqueous phase. The resultant dispersion was frozen with liquid nitrogen for 3 minutes, and then placed in a thermostat at 40℃ for 10 minutes and thawed. This operation was repeated three times. Thereafter, the material thus obtained was frozen with liquid nitrogen, and set in a freeze dryer in which the material was freeze-dried for 30 hours, affording a white mixed lipid powder. 50 mg of this mixed lipid powder was placed in a 10 mL egg-plant type flask, to which 5 mL of biological saline was added. The mixture was stirred with magnet stirrer at room temperature for 2 hours. Then, the resultant mixture was placed in EXTRUDER (trade name, available from Nichiyu, Inc.) having an aperture of 25 mm and was allowed to pass through acetyl cellulose films (available from Fuji Photo Film) having pore sizes of 3.0 μm, 0.45 μm, 0.30 μm and 0.22 μm, respectively, under a pressure of 20 kg/cm^2, thereby preparing a desired vesicle dispersion.

For comparison, vesicle dispersions ware prepared in the same manner as above, except that Compound 5 described at page 26 of the present specification and dipalmitoylphosphocholine were respectively used instead of Compound 3 used above.

Each vesicle dispersion was diluted with a biological

saline to a lipid concentration of 0.075 g/dL. To 1.5 mL of the diluted dispersion, aqueous solutions of polyethylene glycols (PEGs) having various weight-average molecular weights (6 g/dL, 1.5 mL) were added. The critical molecular weight of the polymer at which the aggregation of the vesicles aggregate was caused was determined from the absorbance change at 600 nm measured using a UV-Vis spectrophotometer. Results are shown in Table A below.

Table A: Critical molecular weight of PEG causing the aggregation of the vesiles

Lipid composition (molar ratio)	Critical Molecular Weight
Compound 3/cholesterol/ DPPG (5/5/1)	3,400
Compound 5/cholesterol/ DPPG (5/5/1)	650
Dipalmitoylphosphocholine/ cholesterol/ DPPG (5/5/1)	1,050

As shown in Table A, the addition of PEG having a molecular weight as high as 3,400 was required to cause the aggregation of the vesicles containing Compound 3. The molecular weight of 3,400 is much higher than 1,050 causing the aggregation of

the vesicles containing dipalmitoylphosphocholine, indicating that the vesicle dispersion containing Compound 3 is very stable. By contrast, the vesicles containing Compound 5 aggregated by the addition of PEG having a molecular weight of as low as 650, indicating that the vesicle dispersion containing Compound 5 is very unstable.

I, the undersigned, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Cord and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: March 30, 2005 Keitaro Sou

Keitaro Sou